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BIOSURFACTANT PRODUCTION AND LABORATORY APPLICATION TESTS FOR HEAVY CRUDE OIL

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BIOSURFACTANT PRODUCTION AND LABORATORY APPLICATION TESTS FOR HEAVY CRUDE OIL

by

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Abstract

This paper describes the production and laboratory evaluation of the rhamnolipid PG-201, a biosurfactant, as the primary surfactant for use in chemical EOR of light and heavy crude oil. Surfactant flooding has great potential for oil recovery. Application to some heavy crudes has been difficult due to the high viscosity of the crude and the high cost of surfactant. Biologically produced surfactants, or "biosurfactants" such as PG-201, show considerable promise of lower cost and effective oil recovery as compared to commercial petroleum sulfonates.

This paper summarizes the production of PG-201 from the bacterial strain Pseudomonas aeruginosa from both batch and continuous culture processes. Principal culture parameters for both processes have been investigated for optimal biosurfactant production. Ideal conditions for rhamnolipid production do not necessarily correspond to optimal conditions for bacterial growth nor is surfactant production directly proportional to the growth rate of the microbes.

Analysis of the PG-201 for application in EOR showed that the surfactant behaves as a water soluble surfactant without exhibiting an optimal response to salinity. The biosurfactant showed a strong pH dependence and yielded low interfacial tensions against hexadecane. Oil recovery from corefloods using large pore volumes of 3 percent PG-201 gave the same recovery as equal pore voluems of water-soluble petroleum sulfonates. Recovery was not maintained at either lower concentrations or smaller slug sizes with light oil; however, with heavy crude the oil recovery was maintained at lower surfactant slug sizes.

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Introduction

Recovery of heavy oil reserves is of major importance to many countries throughout the world. Although steamflooding is widely recognized as a successful technique for recovering heavy oil, distribution of this resource indicates that only a fraction of the total is economically recoverable by steam drive. For some countries, such as the United States, steamflooding constitutes 78 percent of the current enhanced oil production (Leonard, 1984). Alternate techniques, such as immiscible CO_2, miscible gas or surfactant flooding, are possible methods for deeper heavy oil recovery. Surfactant flooding of heavy crude has not been used to date due to poor recovery efficiency, marginal cost effectiveness and high initial investment costs.

This paper describes our initial study to recover both light and heavy crude oil via chemical flooding using a biologically produced surfactant. Based upon the good oil recovery observed from corefloods on heavy crude oil, further work to define potential areas of application, thermal limits, and compatibility with conventional surfactants is being conducted. An overview of the biological production and a brief description of the application of the biosurfactant to chemical EOR are presented in this paper.

The use of surfactants in chemical EOR began with sulfonating crude oils or sulfonating alkylated refinery cuts and has evolved to the use of specific synthesized chemicals or chemical blends. Biological systems are untapped sources of surfactants. These systems produce surfactants that are not obtainable from commercial surfactant manufacturers and are generally unavailable from specialty chemical houses. Yet biological systems have been producing these surfactants for millions of years.

Some microorganisms have surface active cell walls or produce compounds with surface active properties. Such microorganisms can be found among the members of the Pseudomonas and Bacillus species, in yeasts of the Candida or Torulopsis species, among fungi such as Penicillium, and in the blue-green algae Spirulina. The surface active compounds of microbial origin can be isolated as cell-bound and/or extracellular. These compounds can be carbohydrates, amino acids, fatty acids, phospholipids, neutral lipids, or combinations of these (Zajic and Seffens, 1984). The hydrophobic or nonpolar part of these molecules usually consists of a fatty acid. The hydrophilic or

polar part of the molecule includes different organic or inorganic groups such as sugars, amino acids, esters, alcohols, acetates or phosphates.

Biosurfactants play a key role in the uptake and degradation of soluble and insoluble substrates by microbes. Hydrocarbons are hydrophobic and are poorly solubilized in water. To oxidize hydrocarbons, the microbe must transport these compounds through its hydrophilic cell wall. Microbes solve this problem by emulsification of the hydrophobic substrate through production of extracellular surfactants or by increasing the lipophilicity of the cell wall through incorporation of surface active compounds such as lipopolysaccharides (Kappeli, 1976). In both cases the result is the same. Substrates are transported or diffused from outside the cell to the cell interior, where the substrate can be used for metabolism by the cell.

The greatest variety of biosurfactants occurs among the glycolipids, where combinations of sugars and lipids with various bonding, structural and ionic states provide a wide range of surfactants with different hydrophilic/lipophilic balances. When the sugar part of the molecule consists of a rhamnose sugar, the resulting glycolipid is a rhamnolipid, such as PG-201 (figure 1). Rhamnolipids have been found to play an important role in the growth of Pseudomonas bacteria on hydrocarbons (Itoh and Suzuki, 1972; Hisatsuka et al., 1971). Traces of rhamnolipid (0.001%) can dramatically stimulate certain bacteria to grow on n-hexadecane.

Production of Rhamnolipids by Microorganisms

The production of rhamnolipids has been investigated in a number of biological reactors. In these experiments, the kinetics of growth, and factors affecting the rate of production of the rhamnolipid have been determined. The objective of these efforts has been to find the composition of nutrient medium and the conditions for optimal cell cultivation, and to eliminate or mitigate production difficulties, such as foaming.

Selected Microorganism

Pseudomonas aeruginosa is the bacterium that produces rhamnolipids. Pseudomonads are Gram-negative, chemoorganotrophic, or facultative chemolithotrophic bacteria and are found in soil and both freshwater and saltwater environments. All species use molecular oxygen as the terminal

electron acceptor in obligatory oxidative metabolism. The cells are polarly flagellated rods of 0.4 to 0.6 by 1.2 microns (Murao et al., 1970). P. aeruginosa has an optimum growth temperature of 37° C. The species is capable of denitrification, i.e., it is able to form gaseous nitrogen and/or other oxides of nitrogen from nitrate or nitrite. This property is used in the elimination of nitrate from waste waters. This bacterium can produce acids from glycerol, xylose and glucose in both aerobic and anaerobic conditions (Murao, 1970). P. aeruginosa is among the most common bacteria to be found in oily locations and has frequently been isolated from industrial oils (Beerstecher, 1954).

Carbon Sources for the Pseudomonas aeruginosa

P. aeruginosa is capable of utilizing different substrates as carbon and energy sources. These compounds range from carbohydrates such as glucose, to hydrocarbons, such as aliphatics, aromatics, or cycloparaffins. Some of these compounds are shown in table 1. It has been reported that n-paraffins or glycerol are the preferable carbon sources for the production of rhamnolipids (Itoh et al., 1971). This comparative study, however, was performed in shake-flasks using a complete nutrient medium, and, as will be discussed later, the medium composition, culture conditions, and type of cultivation can significantly affect the production of rhamnolipids.

Table 1. - Compounds Utilizable by P. aeruginosa as Carbon and Energy Sources

Cyclohexane	Glycerol	Kerosene
n-Dodecane	Heptane	Naphthalene
Gasoline	n-Hexadecane	Paraffin wax
Glucose	Hexane	Tetradecane

(Itoh and Suzuki, 1972; Itoh et al., 1971; Hisatsuka et al.,1971; Beerstecher, 1954)

Production Systems

Batch

In a batch process using a glucose-limited nutrient medium, P. aeruginosa decreased the surface tension and interfacial tension of the medium shortly after the reactor was inoculated with cells (Guerra-Santos et al., 1983). Surface activity of the culture lasted throughout the whole cultivation process. The concentration of rhamnolipid in the culture increased in accordance with produced biomass. The factor cmc (Fcmc), which is proportional to the produced surfactant, reached its maximum value of 8, corresponding with 150 mg/l of rhamnolipid, shortly before the bacterial population stopped increasing (the result of glucose depletion in the medium). The maximum growth rate of P. aeruginosa in this experiment was 0.35 h^{-1}, and the bacteria had a specific oxygen uptake rate of 4-11 mmol $g^{-1}h^{-1}$.

Syldatk and coworkers (1984) used another strain of P. aeruginosa (DSM 2874) and found that the overproduction of rhamnolipid on n-alkanes takes place after the batch process has reached the stationary phase, the phase of microbial growth where all or part of available nutrients are exhausted and the cells are in a relatively inactive metabolic state (Wagner et al., 1983). A further finding was that the nitrogen limitation increases the yield of rhamnolipid from 0.11 to 0.18 g per g n-alkane (Syldatk et al., 1984). However, as the result of such limitation, the cultivation period can be prolonged significantly with a negative effect on the production rate of the cells. Determination of when the rhamnolipid production was initiated (shortly after the inoculation of the cells or in the stationary phase of the growth) could not be established by a simple comparison of the two results. In the first experiment, reduction in surface tension and interfacial tension was taken as the indication of rhamnolipid production; whereas in the second experiment the actual concentrations of glycolipids were measured. Since the critical micelle concentration of glycolipids for lowering the interfacial tension was low (20 mg/l), we conclude that the production of rhamnolipid was initiated at the very beginning of the growth phase.

Continuous Culture

Guerra-Santos and coworkers (1983, Table 2) extended their batch experiments to a continuous type of cultivation and found that the surfactant is produced only at dilution rates close to the maximum growth rate of the bacteria in the batch cultivation. The minimum surface and interfacial tensions of 35 and 5.5 mNm^{-1}, respectively, were higher than the corresponding values of 29 and 0.25 mNm^{-1} that were been obtained in batch cultivations.

In further continuous cultivations, the influence of the nitrogen, iron, and phosphorus content of the medium on the growth and surfactant production have been investigated (Guerra-Santos et al., 1984). The surfactant output of the system could be increased by reducing the concentration of iron relative to the amount of carbon introduced. Further, by maintaining the concentration of nitrogen and phosphorus between certain levels, the productivity could also be increased.

The study of the effect of growth rate on surfactant production showed that under optimum concentrations of nitrogen, phosphorus and iron, good biosurfactant production (Fcmc of 68) takes place at bacterial growth rates less than 0.15 h^{-1} (Guerra-Santos,1984). Higher growth rates caused production of less biosurfactant, whereas higher medium input rates caused more biomass production.

Immobilized and Resting Cells

The findings that overproduction of rhamnolipids takes place in the stationary phase of the growth and the limitation of certain elements can cause such overproduction gave rise to an attempt to separate the growth phase from the production phase. Wagner et al. (1984) have applied this to resting and immobilized cells in production of biosurfactants. For the production of rhamnolipids by resting cells, the cells are first grown on an alkane medium in a batch process. The grown cells are then harvested from the fluid culture and are suspended in a saline solution containing a carbon source without nitrogen. For the production of rhamnolipids by immobilized cells, the cells are first immobilized on a cell-entrapping polymer. The immobilized cells are then used in a semi-continuous, stirred tank reactor (Wagner et al., 1984; Syldatk et al., 1984). The cell-entrapping polymer allows both the substrate and the produced rhamnolipid to pass through the pores. Ca-alginate seems to

be the polymer of choice when glycerin or glucose are used as substrates. However, for n-alkanes, a search is being continued to develop a proper cell-entrapping material.

Comparison of Production Systems

Table 2 compares the production rates of rhamnolipids for different production systems. In the batch system, the highest production rates are obtained using an n-alkane medium with no limitations. Nitrogen limitation in this case causes the prolongation of the cultivation period and a drop in productivity. Rhamnolipid production from resting cells has a better yield compared with systems with growing cells (0.24 g RL per gr substrate against 0.11 g RL per gr substrate, see Syldatk et al., 1984); however, the productivity based on consumed substrate is less (1.3 mg RL $g^{-1}h^{-1}$ against 9.2 mg RL $g^{-1}h^{-1}$). The highest production rate (12.4 mg $g^{-1}h^{-1}$) occurred in a continuous system with optimal medium composition, and it may be expected that even higher production rates could be obtained on n-alkanes.

Operational Problems and Methods of Approach

Because of the anionic characteristics of rhamnolipids and their high water solubility, the system foams thus creating several operational problems. For example, the effective volume of the reactor increases and part of the culture liquid and microorganisms may be lost through the air exhaust line by flotation. Foam escape causes the wetting of the outlet air filters and lines and allows subsequent contamination (Hall et al., 1973). Foam can escape into bearings and other attachments causing deposition of microbes on the lid or reactor walls where the microbes are no longer useful. Extensive foam formation disturbs the reactor homogeneity, which in turn creates an uncontrollable foam pattern due to changing liquid volumes or gas holdup. Foam lowers surfactant production and affects the yield and performance of the entire system.

Two approaches have been taken to solve the foam problem in the process of rhamnolipid production. Fiechter (1978) has developed a bioreactor "COLOR" that consists of a compact short loop with internal circulation. This

TABLE 2. - Rate of Production of Rhamnolipids Based on Carbon Source using Different Cultivation Systems

System	Productivity[a] based on		Reference
	Initial Substrate	Initial Carbon[b]	
Batch[c]			
glucose, no limitation	1.9	4.7	Guerra-S. et t al. 1982
glucose, no limitation	<4.5	<5.3	Syldatk et al., 1984
n-alkane, no limitation	<9.2	<10.8	" " " "
n-alkane, N-limitation	<1.2	<1.4	" " " "
Continuous			
glucose, no limitation	2.2	5.5	Guerra-S. et al., 1982
glucose, optimal N, P and Fe	12.4	31	" " " , 1984
Resting cells[c]			
n-alkane, N-limitation	<1.4	<1.6	Syldatk et al., 1984
n-alkane, N-limitation	<1.3	<1.5	" " " "

(a) Productivity values are based on initial amount of substrate and carbon that has been applied. Values with (<) are based on the amount of substrate and carbon that has been consumed.

(b) Conversion factor for glucose to carbon: 0.40
" " " n-alkane " " 0.85

(c) The lag period and the time required for emptying and recharging the culture vessel between cycles are not included in these calculations.

propeller-equipped bioreactor has very short mixing time with relatively low power input as compared with the classical stirred tank reactor with flat blade turbines (Karrer, 1978) making the total reactor volume available to the culture.

The second solution to minimize foam formation during production of rhamnolipids is the use of the immobilized cell bioreactor. In this system, a carbon source is continuously fed to the reactor, and an adsorption column bypasses the cell-free culture where the produced rhamnolipids are separated. The rhamnolipid-free solution is then recycled to the bioreactor. This system represses the strong foam formation during cultivation of P. aeruginosa (Syldatk, et al., 1984).

Oil Recovery with PG-201

The primary objective of the research was to demonstrate the utility of PG-201 as a primary surfactant. Therefore, initial research concentrated on evaluating PG-201 itself, then in combination with cosolvents such as simple alcohols and then blended with conventional surfactants as necessary.

Chemical Analysis

PG-201, the rhamnolipid produced by P. aeruginosa, is a mixture of anionic surfactants (figure 1) with a composition of 30 percent di-rhamnose and 70 percent mono-rhamnose. The mono-rhamnose molecule has the formula $C_{26}H_{48}O_9$, with a molecular weight of 504 daltons. Assays for the rhamnose moiety determined that the samples were impure (ca 70 percent active) and contained large amounts of other reducing agents, as demonstrated by thin-layer chromatography. A spectrophotometric method specific for methyl pentose was adapted to determine PG-201 concentrations and has been extended to determination of rhamnose in both oleic and aqueous phases (Dische and Schettles, 1948).

Surface Tension and Interfacial Tension and Phase Behavior

Surface tension measurements at ambient temperature on PG-201 (figure 2) showed both a salinity and pH dependence. Interfacial tension (IFT) measurements against alkanes showed a minimum IFT at pH 5 with hexadecane.

The natural pH of PG-201 in brine is pH 5-6. Salinity and concentration were less sensitive variables. A summary pH-IFT scan is shown in figure 3.

We conducted phase partitioning studies on PG-201 with hexadecane as the oleic phase. PG-201 behaves like a typical water-soluble surfactant and solubilized about 2 percent of the oil and 10 percent of the water (figure 4). PG-201 was insensitive to salinity in the range practical for oil recovery; however, below 0.3 percent NaCl the surfactant solubilized nearly all the water. Thus PG-201 has no "optimal salinity". When we conducted phase partitioning studies at constant ionic strength but at various pH's, solutions at pH 8 and above showed persistent macroemulsions. Addition of alcohols to PG-201 raised the IFT and reduced solubilization.

Initial Coreflood Experiments

Corefloods with PG-201 using 25 cm x 3.8 cm diameter fired Berea sandstone cores with an average brine permeability of 250 millidarcies were used to screen formulations of surfactant. Cores were initially brine-saturated, oil-saturated to original oil saturation (S_{oi}) and then waterflooded to residual oil saturation (S_{ow}). Chemical injection of surfactant and/or surfactant-polymer reduced the oil saturation to S_{oc}. Chemical recovery efficiency was calculated as $(S_{ow} - S_{oc})/S_{ow} \times 100$, and reported as R_e for the tertiary oil recovery. Figure 5 illustrates both waterflood and chemical flood recovery on coreflood BS9.

Screening of the PG-201 followed a surfactant evaluation scheme which evaluates initial surfactant slug composition using large pore volumes. Once optimal formulations are obtained, smaller pore volumes are used for recovery tests and surfactant-polymer compatibility tests to develop an effective chemical slug. Table 3 illustrates the tertiary oil recovery efficiency of PG-201 with hexadecane or Bradford crude oil, 41° API. Low concentrations of PG-201 were ineffective with either hexadecane or Bradford crude oil even though IFT's against these hydrocarbons were 52 and 56 millidynes/cm.

Corefloods using buffered solutions designed to maintain constant pH throughout the flood confirmed some of the pH dependence observed with IFT. Based upon IFT, oil recovery would be expected to decline sharply on going from pH 5 to pH 7. Oil recovery using three pore volumes of 3.3 percent PG-201 at pH 5, pH 7, and unbuffered brine solutions which have pH near 7, showed

equivalent oil recovery. A coreflood buffered at pH 8 gave only 20 percent recovery and produced macroemulsions.

Addition of polyacrylamide to the PG-201 surfactant slug to improve mobility control caused an increase in IFT. Addition of Flocon 4800 biopolymer (Pfizer Chemical Company) provided viscosity control over a wide range of salinity without adversely affecting the IFT. Figures 6 and 7 using viscosified surfactant show recovery was not maintained when either lower concentrations (less than 2-3 percent) or smaller pore volumes (less than 2-3) of PG-201 were used.

Before examining blends of PG-201 with conventional surfactants, we recently attempted a flood on heavy oil using a viscosified PG-201 solution. PG-201 showed a slight preference for longer alkanes such as hexadecane in previous alkane carbon scans, and a flood on heavy oil (Wilmington crude, 21° API, 40 cp at 65° C) was attempted. A tertiary oil recovery efficiency of 83 percent was obtained using a 3.2 pore volumes of viscosified 3.3 percent PG-201. The recovery was the same as those obtained with a light oil, even though the IFT was 220 μNm^{-1}. Subsequent floods with smaller pore volumes of PG-201 (figure 8) showed that the recovery efficiency was being maintained. The oil recovered from these corefloods was heavily emulsified but could easily be separated in the laboratory by centrifugation or the addition of chemical emulsion breakers.

Research is continuing to define the limitations on the use of PG-201 for heavy oil recovery as well as screening other biosurfactants for use in chemical EOR to recover both light and heavy crudes.

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TABLE 3. - Initial Flood Tests with PG-201

Test No.	Oil	Brine, % NaCl	Surfactant Conc'n. %	Slug Volume PV	Mobility Buffer PV	S_{oi} %	S_{ow} %	S_{oc} %	Re %
1	hexadecane	5.0	0.5	1.00	2.5	75.5	37.6	35.6	5.3
4	hexadecane	5.0	5.0	0.97	2.9	71.9	39.0	9.7	75.1
6	Bradford	0.5	3.3	3.6	0	73.1	31.6	9.6	69.6
7	Bradford	0.5	0.3	3.1	0	75.9	38.8	36.2	6.7
9	Bradford	0.5	3.3	1.1	0	74.3	35.1	15.5	55.8
13	Bradford	0.5	3.0[a]	3.2	0	76.2	38.2	34.4	10.0

[a]Petrostep 40 (Stepan Chemical Co.)

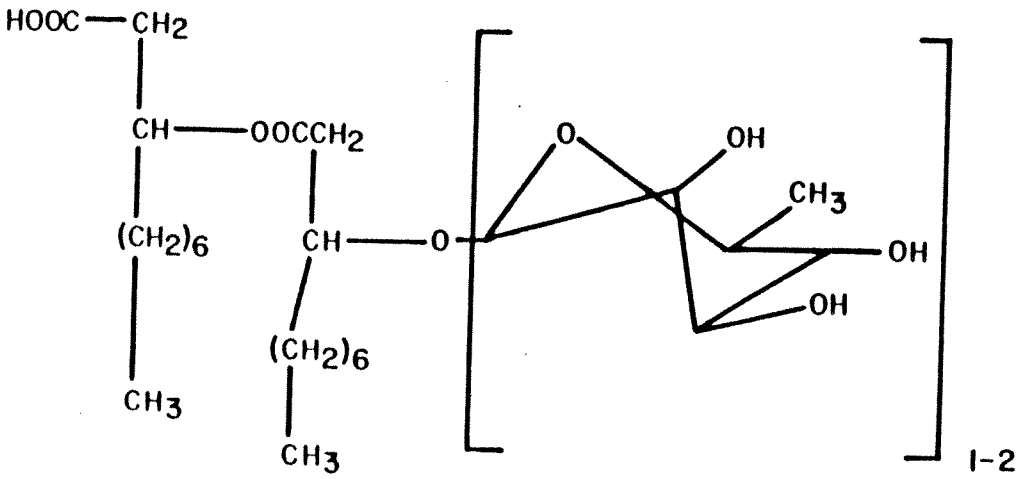


FIGURE 1. - Structure of Rhamnolipid PG-201 produced by Pseudomonas aeruginosa.

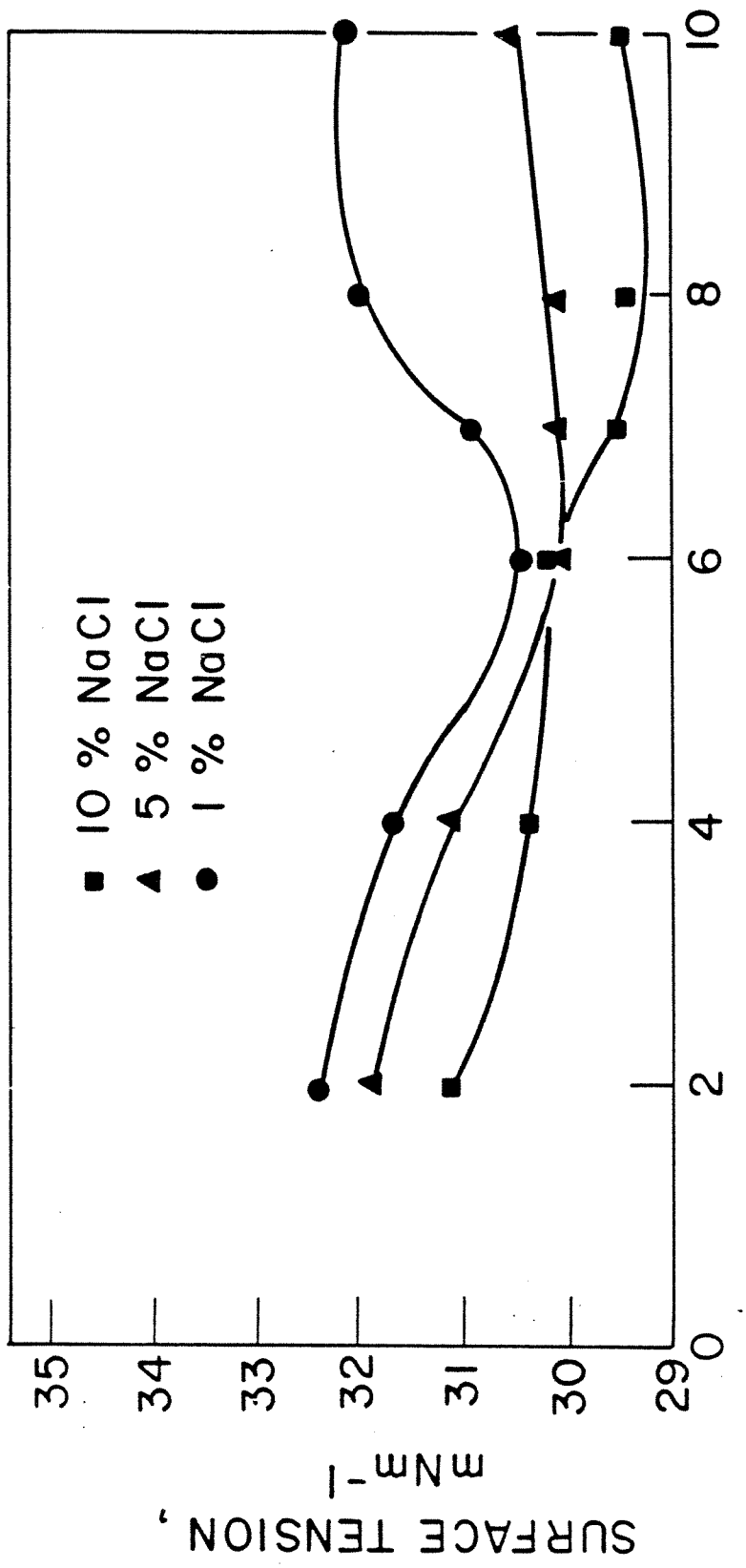


FIGURE 2. - Effect of pH on surface tension of PG-201 (3%).

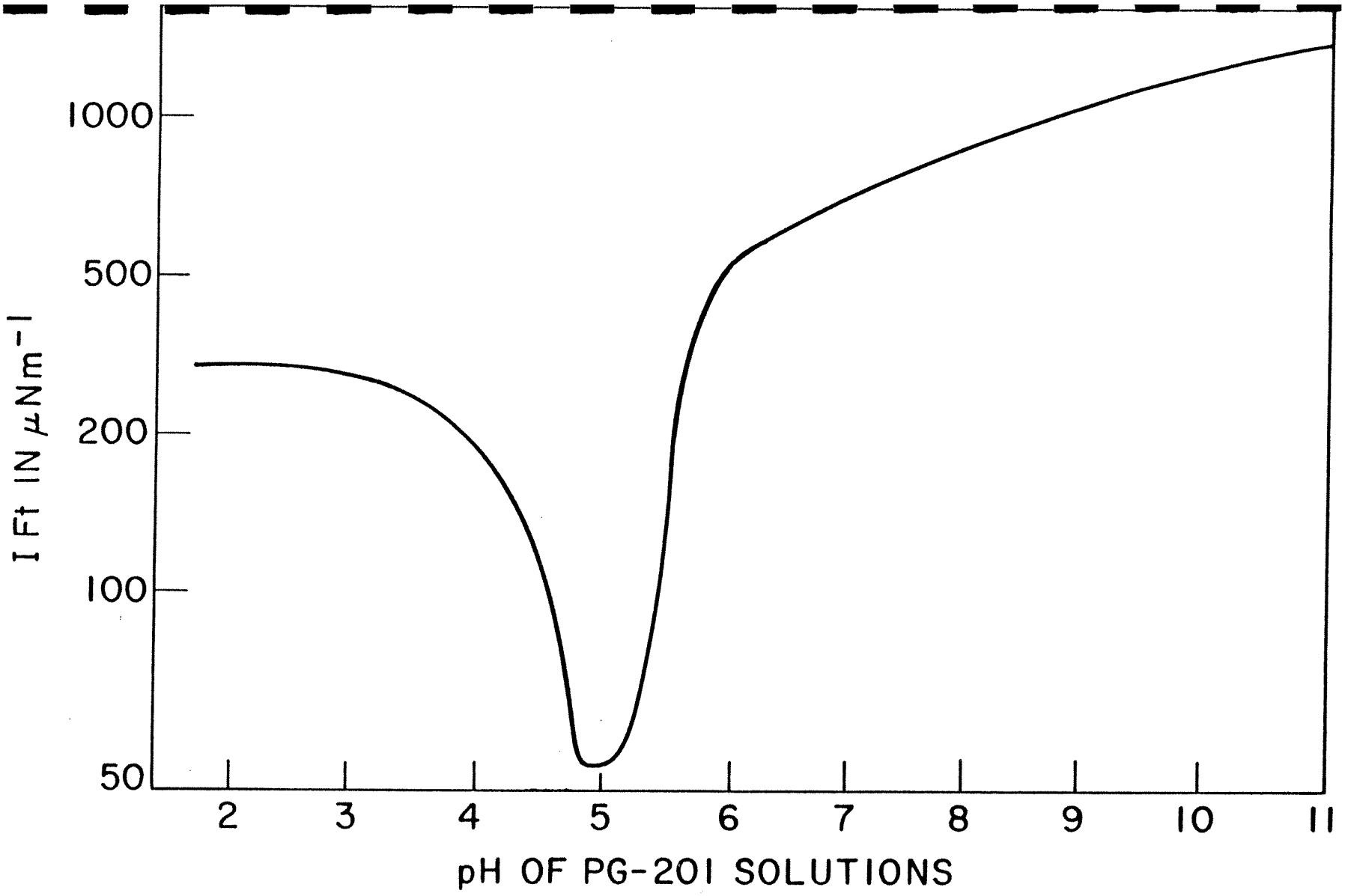


FIGURE 3. - Typical effect of PG-201 (5% in 5% NaCl) on interfacial tension against hexadecane.

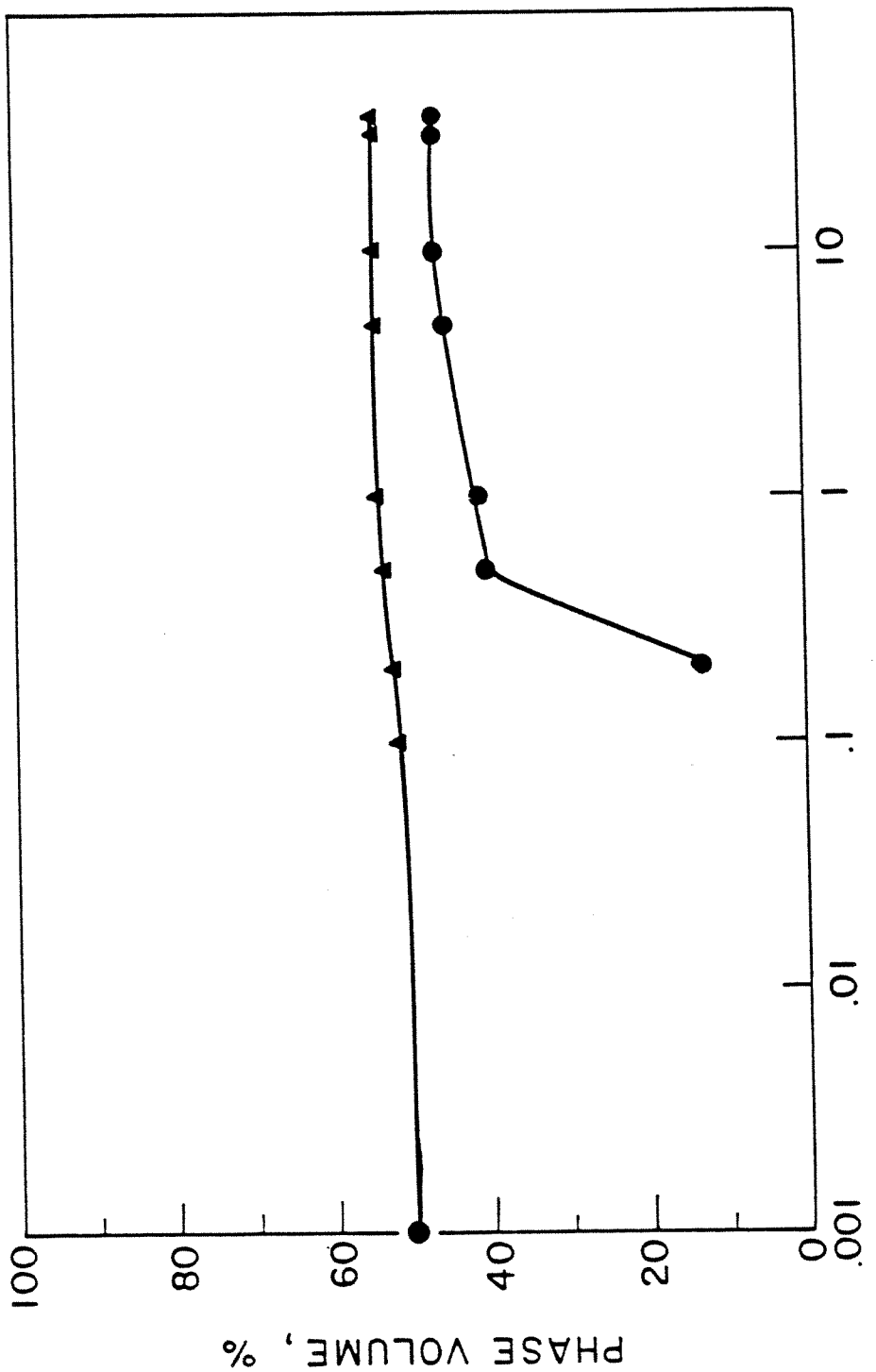
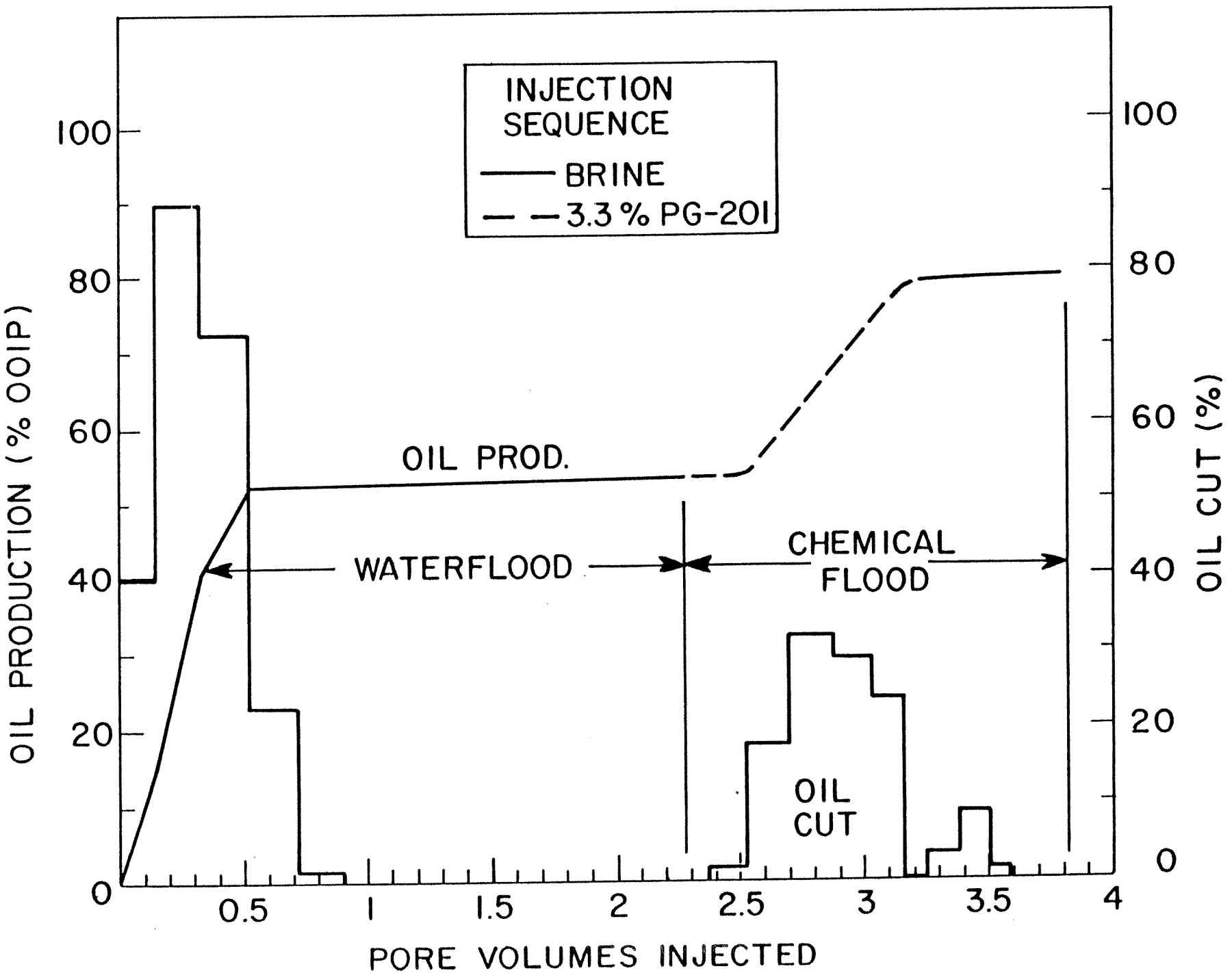


FIGURE 4. - Phase partitioning study of PG-201 (5.6%) with hexadecane.


INJECTION SEQUENCE
BRINE
3.3 % PG-201
OIL PROD.
WATERFLOOD
CHEMICAL FLOOD
OIL CUT
OIL PRODUCTION (% OOIP)
OIL CUT (%)
PORE VOLUMES INJECTED
0
20
40
60
80
100
0.5
1
1.5
2
2.5
3
3.5
4

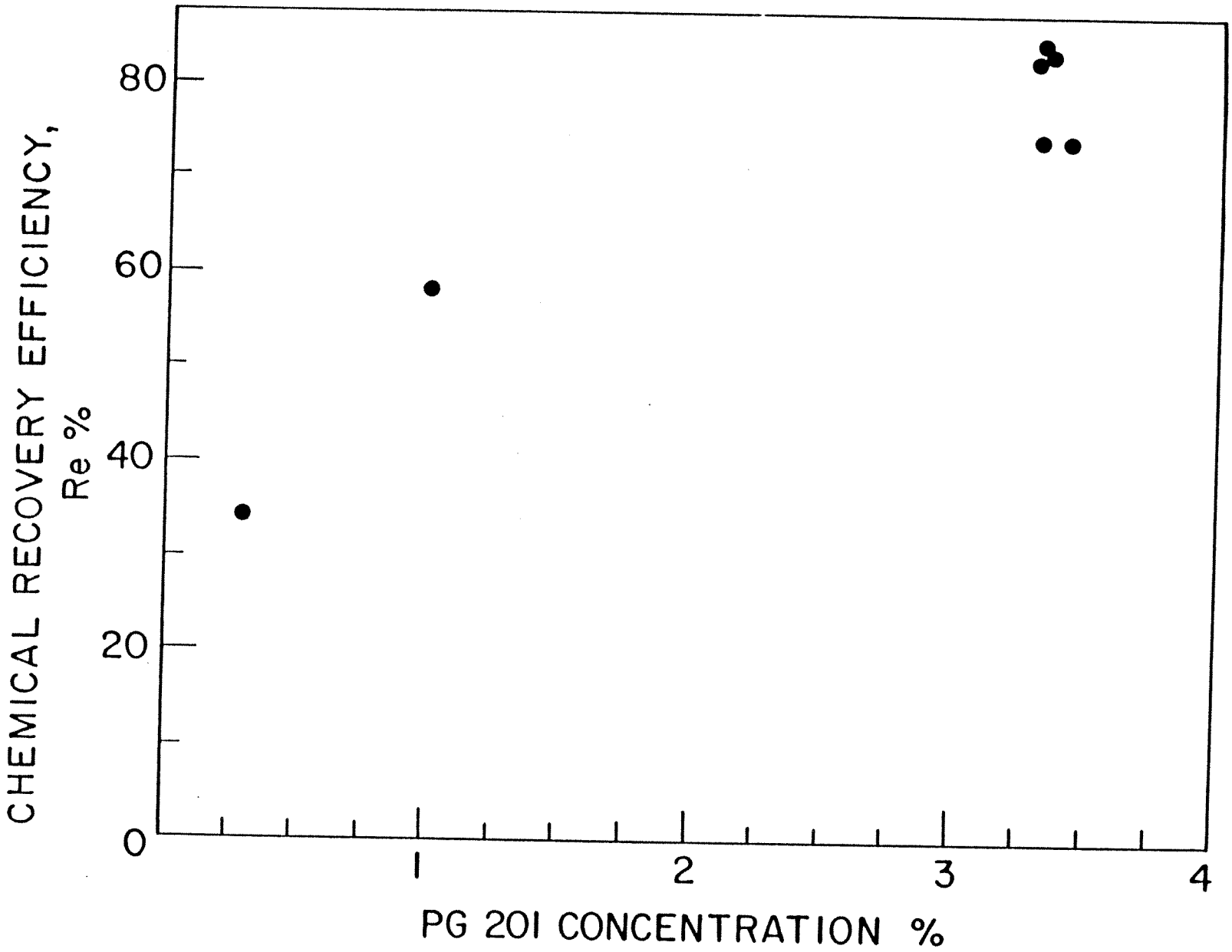


FIGURE 6. - Effect of surfactant concentration on recovery of Bradford crude oil. (Continuous slug injection of surfactant in 0.5% NaCl.)

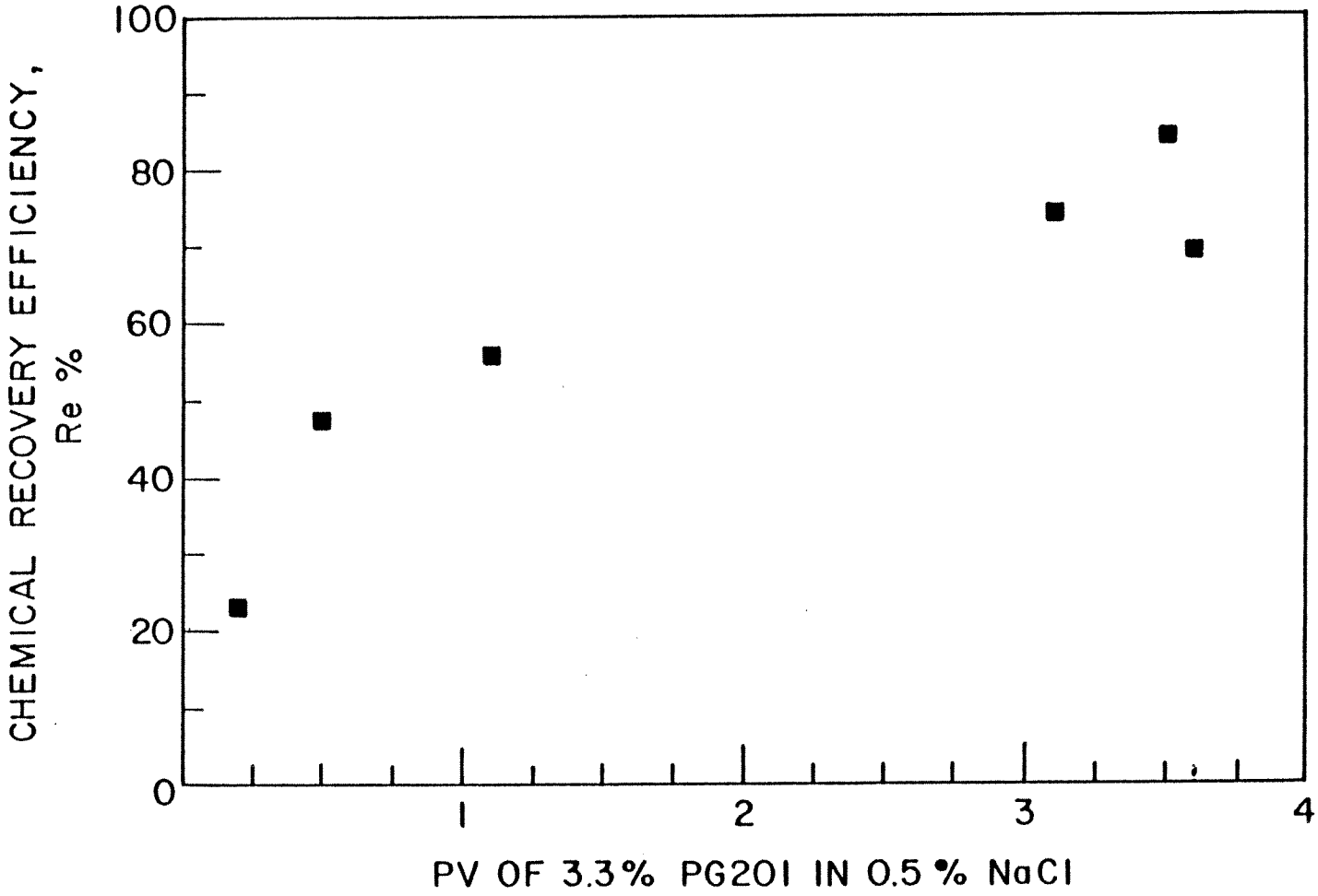


FIGURE 7. - Effect of surfactant slug size on the recovery of Bradford crude oil.

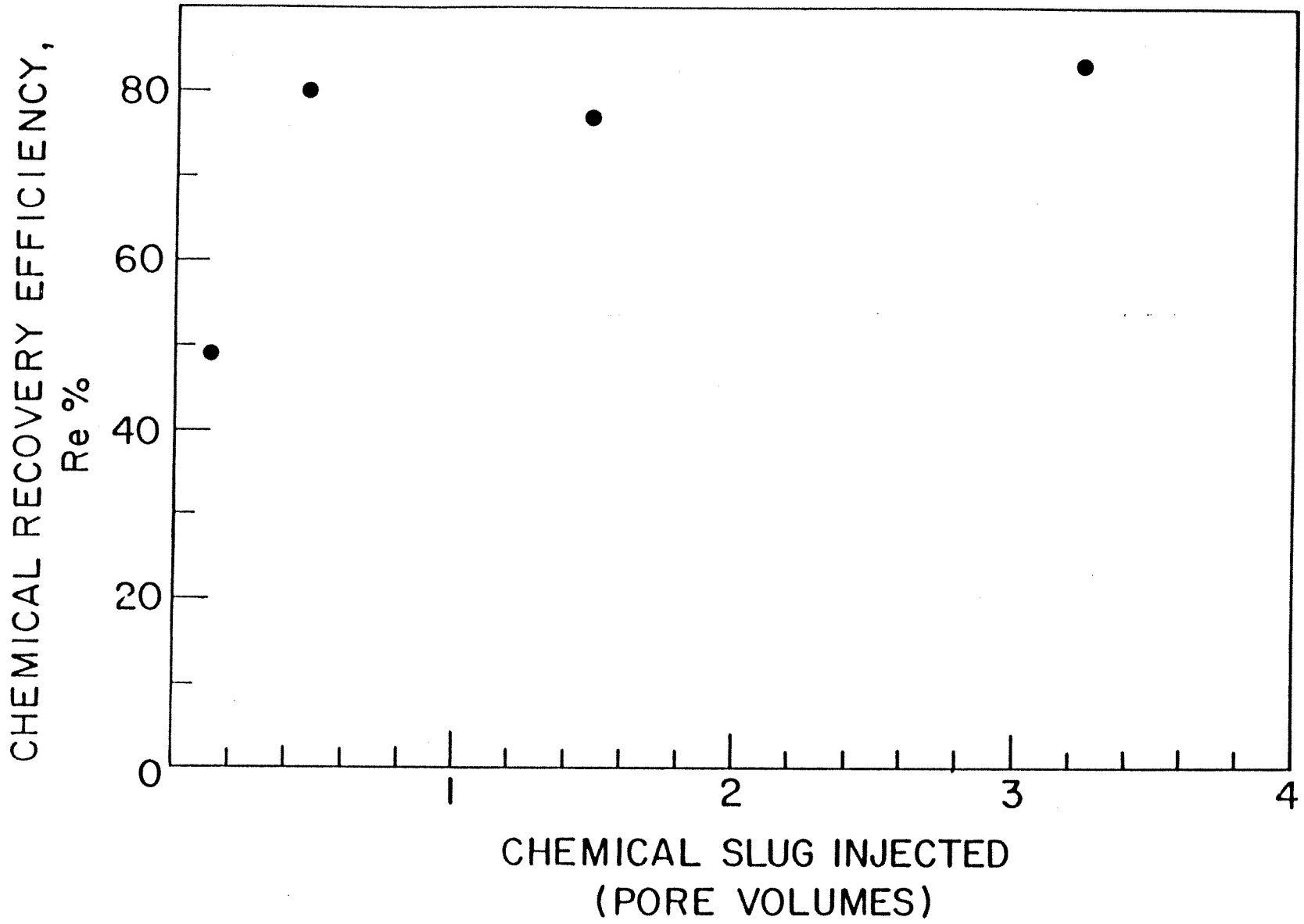


FIGURE 8. - Effect of surfactant slug size on the recovery of Wilmington crude oil. (Surfactant concentration 3.3% PG-201 in 0.5% NaCl.)